

WHAT IS CLAIMED IS:

1. A method for determining an analyte in a sample using an analytical element, the method comprising:

5 providing a mixture by contacting the sample with a binding partner 2 of a specific binding pair 1 (partner 2 of pair 1), and a binding partner 2 of a specific binding pair 2 (partner 2 of pair 2),

adding the mixture to a sample application zone of the analytical element, wherein the element comprises a material enabling liquid transport between the sample application zone and
10 a detection zone located downstream thereof, wherein the detection zone comprises a binding partner 1 of specific binding pair 1 (partner 1 of pair 1) immobilized in such a manner that it is able to bind to the partner 2 of pair 1, and wherein a labeled partner 1 of specific binding pair 2 (partner 1 of pair 2) is present upstream of the detection zone and impregnated on the material such that it can be detached by liquid and is able to bind to the partner 2 of pair 2, and

15 detecting the presence or absence of the label in the detection zone, thereby determining the analyte in the sample.

2. The method of claim 1 wherein the specific binding pair 1 and the specific binding pair 2 independently comprise a pair of specific binding partners selected from the group consisting of
20 a hapten and an antibody, an antigen and an antibody, a lectin and a sugar/saccharide, a ligand and a receptor, avidin/streptavidin and biotin, a nucleic acid and a nucleic acid.

3. The method of claim 1 wherein the partner 1 of pair 2 is an antibody against the partner 2 of pair 2.

4. The method of claim 3 wherein the partner 1 of pair 2 is an antibody against digoxigenin or digoxin.

5 5. The method of claim 1 wherein the partner 1 of pair 2 is labeled with an enzyme or direct label.

6. The method of claim 5 wherein metal or latex particles are used as the direct label.

10 7. The method of claim 1 wherein the partner 1 of pair 2 is located in the sample application zone.

8. The method of claim 5 wherein the partner 1 of pair 2 is located in the sample application zone.

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9. The method of claim 1 wherein an antibody for specific binding with a preselected antigen or hapten is conjugated with the partner 2 of pair 1 and the antibody is conjugated with the partner 2 of pair 2.

20 10. The method of claim 1 wherein an antigen, hapten or oligopeptide is conjugated with the partner 2 of pair 1 and the antigen, hapten or oligopeptide is conjugated with the partner 2 of pair 2, wherein the antigen, hapten or oligopeptide specifically binds to a preselected antibody.

11. The method of claim 1 wherein the partner 2 of pair 1 and the partner 2 of pair 2 are present in separate containers, wherein the separate containers do not include the analytical element.
- 5 12. The method of claim 1 wherein the partner 2 of pair 1 and the partner 2 of pair 2 are stored together in one container, wherein the container does not include the analytical element.
13. The method of claim 1 wherein the partner 2 of pair 1 is conjugated to a nucleotide, oligonucleotide, a nucleic acid, an antibody, a hapten or antigen or an epitope representing an
10 antigen or a lectin or a receptor for a ligand.
14. The method of claim 13 wherein the partner 2 of pair 1 is biotin.
15. The method of claim 1 wherein the partner 2 of pair 2 is conjugated to a nucleotide,
15 oligonucleotide, a nucleic acid, an antibody, a hapten or antigen or an epitope representing an antigen or a lectin or a receptor for a ligand.
16. The method of claim 15 wherein the partner 2 of pair 2 is a hapten.
- 20 17. The method of claim 16 wherein wherein the hapten is digoxigenin or digoxin.
18. A method for determining the presence of an analyte using an analytical element comprising a material enabling liquid transport between a sample application zone where a

sample is applied and a detection zone located downstream thereof, wherein the detection zone comprises a binding partner 1 of specific binding pair 1 (partner 1 of pair 1) immobilized in such a manner that it is able to bind to a binding partner 2 of specific binding pair 1 (partner 2 of pair 1), and wherein a labeled partner 1 of specific binding pair 2 (partner 1 of pair 2) is present
5 upstream of the detection zone and impregnated on the material such that it can be detached by liquid and is able to bind to a specific binding partner 2 of specific binding pair 2 (partner 2 of pair 2); the method comprising:

adding to the sample the sample application zone a substance derived from and representing the analyte wherein the substance comprises partner 2 of pair 1 and partner 2 of
10 pair 2,

moving said substance by liquid transport in the analytical element towards the detection zone; and

binding said substance to partner 1 of pair 1 in the detection zone; and
detecting the labelled partner 1 of pair 2 bound in the detection zone.

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19. The method of claim 18 wherein the substance derived from and representing the analyte is formed by adding to the analyte an antibody wherein part of the antibody carries partner 2 of pair 1 and the other part of the antibody carries partner 2 of pair 2.

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20. The method of claim 18 wherein the substance derived from and representing the analyte is formed by adding to the analyte an antigen, hapten or oligopeptide wherein a part of the antigen, hapten or oligopeptide carries partner 2 of pair 1 and the other part of the antigen, hapten or oligopeptide carries partner 2 of pair 2.

21. The method of claim 18 wherein the analyte is a nucleic acid which is amplified, whereby partner 2 of pair 1 or partner 2 of pair 2 is bound to a nucleotide or to an oligonucleotide that is incorporated into the amplification product of said nucleic acid, and the amplification product is hybridized with a complementary nucleic acid having partner 2 of pair 1 or partner 2 of pair 2 bound thereto, provided that when the amplification product has partner 2 of pair 1 bound thereto, the complementary nucleic acid has partner 2 of pair 2 bound thereto and when the amplification product has partner 2 of pair 2 bound thereto, the complementary nucleic acid has partner 2 of pair 1 bound thereto.

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22. The method of claim 18 wherein the analyte is a nucleic acid and said substance comprises the nucleic acid hybridized with two nucleic acid probes one of which contains partner 2 or pair 1 and the other contains partner 2 of pair 2.

15 23. An analytical element for determining the presence of an analyte, the element consisting essentially of a material enabling liquid transport between a sample application zone where a sample is applied and a detection zone located downstream thereof, wherein the detection zone contains a partner 1 of a specific binding pair 1 (partner 1 of pair 1) immobilized in such a manner that it is able to bind to a partner 2 of the specific binding pair 1 (partner 2 of pair 1) when the partner 2 of pair 1 contacts the partner 1 of pair 1, wherein the partner 2 of pair 1 is not the analyte, and wherein a labeled partner 1 of a specific binding pair 2 (partner 1 of pair 2) is present upstream of the detection zone and impregnated on a material such that it can be detached by liquid and is able to bind to a partner 2 of the specific binding pair 2 (partner 2 of

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pair 2) when the partner 2 of pair 2 contacts the partner 1 of pair 2, and the partner 2 of pair 2 is not the analyte, wherein both the partner 2 of pair 1 and the partner 2 of pair 2 are (i) not impregnated or immobilized on the material and (ii) added to the sample to bind the analyte before the sample is applied to the application zone.